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The relationship between interferon gamma gene expression and cytomegalovirus infection in women at risk of spontaneous abortion in Dhi Qar Governorate

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Abstract:

he study aims to investigate the mechanisms of cytokines, particularly interferon-gamma (IFN-γ), in pregnant women exposed to cytomegalovirus (CMV) with recurrent spontaneous miscarriages (RSM). Thus, 38 blood samples from the above women were collected and compared with 14 samples from healthy participants. Half of the sample volume was transferred to an EDTA tube (2 mL), and the remaining half to a gel tube (3 mL) for centrifugation to separate the serum. The age of the subjects ranged from 15 to 47 years. In addition, the IgM and IgG antibodies were detected in all serum samples with ELISA. The IFN-γ samples were tested by molecular methods to determine the gene expression of interferon-γ in women with RSM compared with controls. ELISA results: one individual (2.63%) tested positive for anti-HCMV IgM antibodies, 33 women (86.84%) tested positive for anti-HCMV IgG antibodies, and four women (10.52%) were found to be positive for both IgM and IgG antibodies. Notably, none of the women in the study group tested negative for both antibodies. The IFN-γ gene was assessed by real-time PCR analysis of DNA extracted from IFN-γ samples; expression in peripheral blood MCs was decreased in women with RSM compared to controls. This lower IFN-γ expression suggests a less effective antiviral immune response, capable of confining the virus's spread within the host.

Keywords: HCMV, miscarriage, IFN-γ, RT-PCR, ELISA, IgM, IgG

1. Introduction

Interferons (IFNs) are cytokine that is essential for defending against viral infections. nfected cells mainly produce type I interferons (IFN-α and IFN-β). T lymphocytes and natural killer cells mainly produce type II interferon (IFN-γ) (NK) cells. IFN-γ plays a vital role in determining immune responses. It stimulates macrophages, enhances antigen presentation, and influences processes such as cell development and death. IFN-γ interacts with other cytokines to influence the expression of about 200 genes [1].

The immune system in the uterus must be tightly regulated to promote a successful pregnancy. It creates a balance in which the body can accept the semi-allogeneic fetus while simultaneously fighting off infection. However, immune stability, which arises from interactions among innate lymphocytes, T cells, and dendritic cells, is more complex. To maintain tolerance and controlled inflammation, these cell forms collaborate [2]. NK cells very obviously drive the immune balance between a mother and her fetus [3].

Various factors can negatively affect pregnancy outcomes. Factors like advanced maternal age, environmental toxins, and unhealthy lifestyles [4] contribute to the risk. Implantation failure may occur because of abnormal conditions of the uterus, such as chronic endometritis and thin endometrium. Structural conditions, including a uterine septum, are prevalent in infertile women and in women who have undergone cesarean section [5]. Pregnancy success relies on a complex network of cytokines, immune cells, and regulatory pathways, and deviations in this system, including altered placental immunity or immune cell function, correlate with recurrent miscarriage [6].

Recurrent spontaneous miscarriages (RSM) are defined as three or more consecutive pregnancy losses prior to 20 to 28 weeks of gestation [7]. Many factors can contribute to RSM, including abnormal chromosomes or genes, maternal age, and infections. Among potential infectious causes, increased risk of RSM has been reported following viral infections, particularly cytomegalovirus (CMV) [8]. CMV infection, when it occurs in pregnancy, poses serious complications for fetal development and may lead to fetal malformations of the brain, growth restriction, or fetal death. Despite having had a prior CMV infection, a woman remains at risk of reinfection, as prior infection does not confer sufficient immunity or prevent viral reactivation. Testing the mother's blood for CMV-specific IgG and IgM antibodies is the primary method for diagnosing CMV infection in pregnant women [9].

Research shows that cytokine imbalances during pregnancy have an ambiguous effect on pregnancy outcomes for women who have CMV infections and suffer repeated miscarriages. The essential role of IFN-γ in viral defence and immune control suggests that alterations in its levels could compromise the effectiveness of the maternal immune response and lead to pregnancy termination. The research intends to study IFN-γ production in pregnant women with recurrent miscarriage due to CMV infection while evaluating their levels against those of normal pregnant women. The study hypothesizes that women who experience recurrent miscarriage from CMV infection will demonstrate reduced IFN-γ levels compared to controls, thus indicating its essential role in pregnancy maintenance and viral control.

2. Materials and Methods

Study Design and Sample Collection

The study analyzed 38 women who experienced spontaneous miscarriages and 14 healthy women who formed the control group. The study obtained samples at Bint Al-Huda Teaching Hospital in Thi Qar City from September 2024 to February 2025. Each participant provided venous blood, which was separated into two portions: one placed in a gel tube for serological testing and the other transferred to an EDTA tube for molecular testing. The CMV IgM & IgG enzyme immunoassay kit from CAMP MEDICA Group in Romania was used to test serum samples obtained from blood spun in gel tubes for cytomegalovirus (CMV)- specific IgM and IgG antibodies, according to the manufacturer's instructions.

Molecular Analysis

Interferon-gamma (IFN-γ) gene expression was determined through real-time quantitative polymerase chain reaction (qPCR). Blood samples stored in EDTA tubes provided RNA, which we then converted to cDNA using reverse transcription. The qPCR reactions required GoTaq® qPCR Master Mix from Promega USA as a reagent. Real-time detection of amplification products was performed using fluorescent probes, which were monitored on a Stratagene Real-Time Thermal Cycler P, USA. The cycling conditions were as follows: an initial enzyme activation

step at 95°C for 2 minutes, followed by 40 cycles that included denaturation at 95°C for 15 seconds, annealing at 60°C for 1 minute, and extension at 60°C for 1 minute. The IFN-γ gene expression analysis employed primers that were created from the NM_000619 accession sequence and synthesized through ORIGENE.

Table 1. The primer sequences.

Primer	Sequence (5'→3')	Length (bp)	Amplicon Size (bp)
Forward	GAGTGTGGAGACCATCAAGGAAG	23	124
Reverse	TGCTTTGCGTTGGACATTCAAGTC	23	

3. Results

Age Distribution of CMV Seropositivity

The age distribution of cytomegalovirus (CMV) seropositivity among the study group women is presented in Table 2. The data indicate that younger women had slightly higher CMV seroprevalence than their older counterparts. The percentage of seropositive cases among women between ages 15 and 30 reached 52.63% (20 out of 38). Among seropositive cases, women aged 30-45 accounted for 47.36% (18 of 38).

Table 2. Distribution of HCMV seropositivity among study participants by age group.

Age group	Study group No.	Percentage %
15-30 years	20	52.63%
30-45 years	18	47.36%
Total	38	

Serological Profiles of Anti-HCMV Antibodies

Table 3 presents the serological profiles of anti-Human Cytomegalovirus (HCMV) IgM and IgG antibodies in both the study and control groups. Only one woman among the 38 who experienced spontaneous miscarriage (2.63%) showed positive results for anti-HCMV IgM antibodies. The majority of these women (86.84%) showed positive results for anti-HCMV IgG antibodies. Four women, representing 10.52%, tested positive for both IgM and IgG antibodies. The study group of women demonstrated complete antibody positivity, as none tested negative for both IgM and IgG antibodies. The entire control group of 14 women displayed negative results for both IgM and IgG antibodies.

Table 3. Serological profile of anti-human cytomegalovirus (HCMV) IgM and IgG antibodies in study and control groups.

Group	Anti-HCMV IgM	%	Anti-HCMV IgG	%	Anti-HCMV IgM and IgG	%	Negative (HCMV) IgG and IgM	%	Total sample
Study group	1	2.63	33	86.84	4	10.52	0	0	38
Control group	0	0	0	0	0	0	14	100	14

Interferon-γ Gene Expression Analysis

The $2^{-\Delta\Delta CT}$ method was applied to measure gene expression after using GAPDH [10]**.** Data showed that women experiencing recurrent miscarriage exhibited significantly lowered interferon-γ expression when compared to women who had normal pregnancies. Interferon-γ levels in the patient group averaged 0.006126 ± 0.002883, which was statistically lower than the control group average of 0.01814 ± 0.006480 ($P < 0.0001$), as detailed in Table 3. IFN-γ expression was reduced by 2.6-fold in the miscarriage group compared to the healthy control group (Figure 2, $p = 0.0001$).

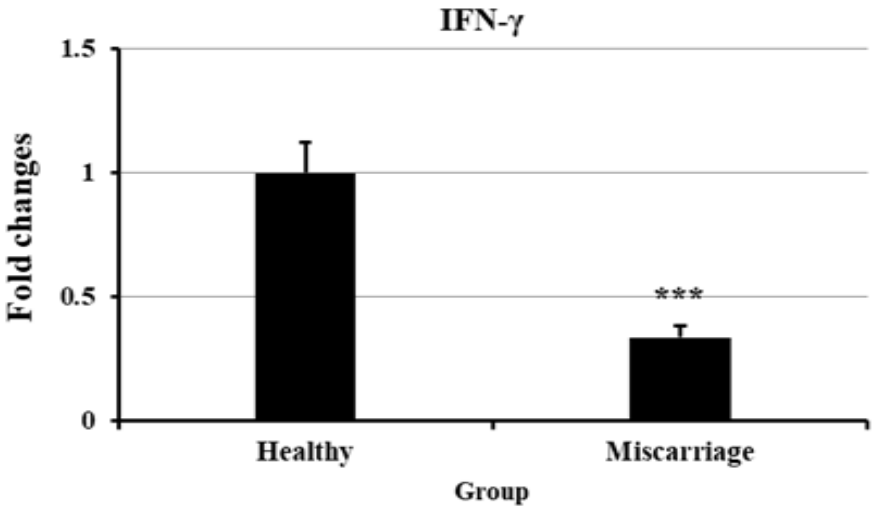

Figure 2. Comparison of interferon-γ (INF-γ) gene expression levels between healthy women and women with recurrent miscarriage. The bar graph shows the fold change of INF-γ, with the healthy group exhibiting significantly higher expression than the miscarriage group ($P < 0.0001$). Error bars represent standard deviation. Statistical significance was determined using the Mann-Whitney test.

4. Discussion

The research investigated how human cytomegalovirus (HCMV) infection and recurrent spontaneous miscarriage affect immune responses in women of reproductive age. The study revealed that HCMV seropositivity rates differed substantially between women who experienced recurrent miscarriages and those without a miscarriage history, while immune parameters showed significant alterations, particularly in interferon-γ (INF-γ) expression.

The data analysis revealed that younger women aged 15 to 30 accounted for 52.63% of HCMV-seropositive cases, compared with 47.37% in the 30 to 45 age group. The minor difference in seropositivity between age groups might indicate that younger women face greater exposure risk. The difference may result from social and behavioral variables, such as more contact with children in daycare settings or different immune system responses between age groups. Research from the past has shown comparable findings where age became an important determinant for HCMV infection rates throughout reproductive years [11].

The serological analysis revealed that 86.84% of women in the study group tested positive for anti-HCMV IgG, indicating past infection and the latent presence of the virus. A small percentage (2.63%) showed positive IgM results, indicating an ongoing or recent infection. A total of 10.52% of participants showed simultaneous positivity for both IgM and IgG antibodies. The control group showed a complete absence of IgG and IgM antibodies among all members, indicating no past exposure. The distinct difference between groups suggests a direct connection between HCMV infection and recurrent miscarriage. The results support those of [12], who observed elevated IgG and reduced IgM antibody levels in women with recurrent pregnancy loss. The findings from this study contradict those of [13], who reported that toxoplasmosis and rubella infections had higher associations with miscarriage than HCMV and HSV-II infections. The observed discrepancies might result from differences in regional patterns, testing approaches, and patient demographics.

The molecular tests revealed a significant decrease in INF-γ levels in the study participants compared with the control group (0.006126 ± 0.002883 vs. 0.01814 ± 0.006480, $p < 0.0001$), indicating reduced immune activity in women with recurrent miscarriages. As a key component of antiviral immunity, INF-γ activates macrophages to enhance antigen presentation. INF-γ deficiency in the body may result in reduced HCMV clearance, allowing the virus to remain active and potentially causing pregnancy complications. The results of this study align with those of [11], who demonstrated impaired immune responses in women who transmitted HCMV to their fetuses. The findings from this study oppose the results obtained by [14], who detected elevated cytokine amounts in women with repeated miscarriages. The observed discrepancies in results might stem from differences in the methods used to measure cytokines, the timing of sample collection, and the specific cytokine targets assessed in each study.

The findings demonstrate that HCMV screening needs implementation for women who experience repeated miscarriages, particularly in populations with elevated risk. The research shows that latent HCMV infection occurs frequently among participants because IgG antibodies are prevalent throughout the study population, which may lead to adverse pregnancy outcomes. Monitoring immune function through the Th1/Th2 cytokine balance, along with INF-γ levels, could help predict which individuals are at higher risk of HCMV-related complications. Implementing educational programs that focus on minimizing HCMV exposure during pregnancy would substantially decrease the number of infections. Future research needs to conduct extended investigations to establish if HCMV reactivation presents a direct risk for fetal demise while also evaluating antiviral and immune-modulating treatment efficacy.

This research study faces two main limitations: a small sample size and a cross-sectional design that limit its ability to draw causal conclusions. The study did not evaluate simultaneous TORCH infections, which may create difficulties in establishing direct links between HCMV and miscarriage. The research limited its cytokine analysis to INF-γ alone, so a comprehensive evaluation of immune parameters would enhance our understanding of immune dysfunction in these conditions.

5. Conclusion

The research demonstrates a significant link between human cytomegalovirus infection and repeated pregnancy losses. The study observed increased IgG antibody levels together with reduced interferon-γ concentrations in women who experienced pregnancy loss. These results indicate that a latent HCMV infection and a weaker immune response might play a role in miscarriages. Women who have elevated risk factors for adverse pregnancy outcomes should undergo routine HCMV testing combined with immune function assessments to reduce adverse pregnancy events. Further analysis of extensive patient samples, along with comprehensive immune analysis, will validate these results and inform the development of preventive measures.

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Conflict of Interest

The authors declare that they have no conflicts of interest relevant to the content of this manuscript.

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